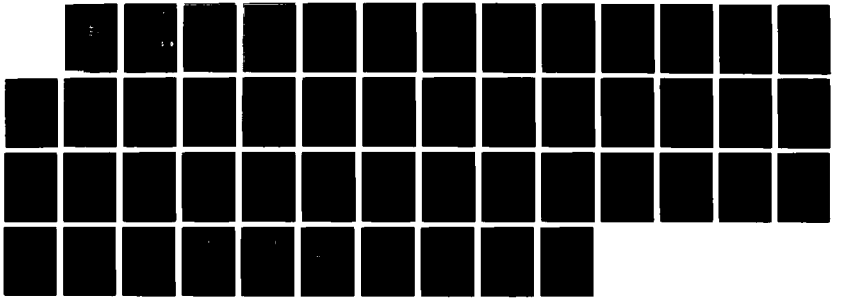
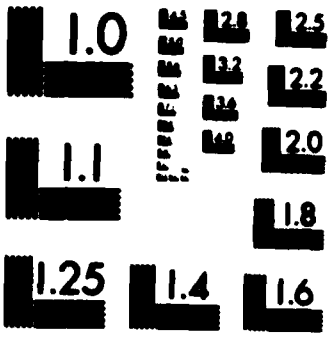
AD-A101 471 EFFECT OF ORGANOPHOSPHATE COMPOUNDS ON RENAL FUNCTION AND TRANSPORT(U) NEBRASKA UNIV MEDICAL CENTER OMAHA DEPT OF PHARMACOLOGY W O BERNDT 15 SEP 83 1/1

UNCLASSIFIED DAMD17-82-C-2220 F/G 6/11 NL





MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963-A

AD _____

EFFECT OF ORGANOPHOSPHATE COMPOUNDS ON RENAL FUNCTION AND TRANSPORT

ANNUAL REPORT

WILLIAM O. BERNDT

15 SEPTEMBER 1983

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-82-C-2220

University of Nebraska Medical Center
Department of Pharmacology
42nd and Dewey Avenue
Omaha, Nebraska 68105

AD-A181 471

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER

2. GOVT ACCESSION NO.

3. RECIPIENT'S CATALOG NUMBER

4. TITLE (and Subtitle)

Effect of Organophosphate Compounds on Renal Function and Transport

5. TYPE OF REPORT & PERIOD COVERED

Annual: 1 Sept. 82-31 Aug. 83

6. PERFORMING ORG. REPORT NUMBER

7. AUTHOR(s)

William O. Berndt, Ph.D.

8. CONTRACT OR GRANT NUMBER(s)

DAMD 17-82-C-2220

9. PERFORMING ORGANIZATION NAME AND ADDRESS

Department of Pharmacology
University of Nebraska Medical Center
42nd and Dewey Avenue, Omaha, NE 68105

10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS

11. CONTROLLING OFFICE NAME AND ADDRESS

U.S. Army Medical Research and Development Command
Fort Detrick, Frederick, Maryland 21701-5012

12. REPORT DATE

15 Sept. 83

13. NUMBER OF PAGES

49

14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)

15. SECURITY CLASS. (of this report)

No classification

15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release;
distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

organophosphate compounds
cholinesterase inhibitors
diisopropyl fluorophosphate
DFP
renal function
renal PAH transport
renal TEA transport
renal organic ion transport

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The present program was undertaken to examine whether or not organophosphate cholinesterase inhibitors have effects on renal function and transport in the rat. These studies were proposed because of several suggestions in the scientific literature that cholinergic agents may have direct effects on renal transport above and beyond effects exerted through changes in renal blood flow. The organophosphate inhibitors are particularly suitable because of their long lasting effects. → over

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

A

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

Block 20:

To date, two types of experiments have been undertaken. First, studies ~~were undertaken~~ in unanesthetized rats to examine the effects of diisopropyl fluorophosphate (DFP) on overall renal function. Animals were given single subcutaneous doses of DFP ranging from 1 to 4 mg/kg, placed in metabolism cages, and renal function followed for several days. These studies indicated that DFP caused increased urine flow, a decreased osmolality of the urine, an increased sodium excretion, and an increased excretion of protein, glucose and blood. No effects were observed on the excretion of potassium. The effects were of a transient nature with a return to the control status by 24 hours. The effects observed did not correlate well with inhibition of renal cholinesterase.

Clearance studies on anesthetized animals confirmed the results on the unanesthetized animals. In the clearance experiments, inulin was used to monitor glomerular filtration rate and an electromagnetic flow meter was used to monitor renal blood flow. Although at low doses GFR and renal blood flow increased, this effect was not sustained and at high doses decreased renal blood flow was observed while urine flow was sustained at high rates.

The second major type of experiment undertaken involved the use of renal cortex slices. These studies were done to determine whether or not direct effects of organophosphate compounds on renal slice function could be observed. DFP was found to inhibit the transport of p-aminohippurate (PAH) whether administered in vivo or added to slices in vitro, while effects on the transport of the cation tetraethylammonium were produced less regularly.

B

Summary

The present program was undertaken to examine whether or not organophosphate cholinesterase inhibitors have effects on renal function and transport in the rat. These studies were proposed because of several suggestions in the scientific literature that cholinergic agents may have direct effects on renal transport above and beyond effects exerted through changes in renal blood flow. The organophosphate inhibitors are particularly suitable because of their long lasting effects.

To date, two types of experiments have been undertaken. First, studies were undertaken in unanesthetized rats to examine the effects of diisopropyl fluorophosphate (DFP) on overall renal function. Animals were given single subcutaneous doses of DFP ranging from 1 to 4 mg/kg, placed in metabolism cages, and renal function followed for several days. These studies indicated that DFP caused increased urine flow, a decreased osmolality of the urine, an increased sodium excretion, and an increased excretion of protein, glucose and blood. No effects were observed on the excretion of potassium. The effects were of a transient nature with a return to the control status by 24 hours. The effects observed did not correlate well with inhibition of renal cholinesterase.

Clearance studies on anesthetized animals confirmed the results on the unanesthetized animals. In the clearance experiments, inulin was used to monitor glomerular filtration rate and an electromagnetic flow meter was used to monitor renal blood flow. Although at low doses GFR and renal blood flow increased, this effect was not sustained and at high doses decreased renal blood flow was observed while urine flow was sustained at high rates.

The second major type of experiment undertaken involved the use of renal cortex slices. These studies were done to determine whether or not direct effects of organophosphate compounds on renal slice function could be observed. DFP was found to inhibit the transport of p-aminohippurate (PAH) whether administered in vivo or added to slices in vitro, while effects on the transport of the cation tetraethylammonium were produced less regularly.

FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

TABLE OF CONTENTS

Progress Report

Statement of Problem and Background.

This project was designed to characterize possible effects of cholinesterase inhibitors (i.e., the organophosphate compounds) on renal function and transport in the rat. The studies were proposed because of several suggestions in the scientific literature that the cholinergic nervous system may plan an important role in renal function and transport (Barajas, 1978; Carter, 1971). Although cholinergic agents have effects on the kidney, studies have not been undertaken to examine organophosphate cholinesterase inhibitors for their direct effects on transport or transport functions.

The initial investigations proposed were to have involved an assessment of the effects of organophosphate compounds on fluid and electrolyte balance in the rat. Originally, it was proposed that these studies would be done with diisopropyl fluorophosphate (DFP) as well as several more reactive substances, pinacolyl methylphosphorofluoridate (soman), isopropylmethylphosphorofluoridate (sarin), and ethyl n-dimethylphosphoroamidocyanidate (tabun). Both single and multiple dose regimens of the test compounds were to be studied and the results of the whole animal experiments were to be related to effects on renal function produced in anesthetized rats. The latter experiments were planned in order to permit quantitative assessment of renal blood flow and glomerular filtration rate as well as urinary electrolyte excretion.

Finally, to assess whether or not these compounds might have direct effects on renal transport, a series of in vitro experiments were planned. These studies, initially, were to involve the use of renal cortex slices and the effects of the organophosphate compounds on the transport of model organic anions and cations were to be investigated. Should effects be observed with the in vitro experiments, evidence would have been obtained to suggest a direct effect of the organophosphate compounds on a tissue transport function as opposed to effects of these substances mediated through alterations in renal hemodynamics. Of course, all of the above studies were to be correlated with changes in cholinesterase activity of the kidney.

Results. The first studies undertaken were those related to the alteration of renal function in the unanesthetized rat by DFP. In conjunction with these studies, the effects of DFP on various renal function parameters in the anesthetized rat also were examined. Both of these studies are well along and a manuscript has been submitted for publication (see appendix). Because the data are summarized in great detail in the enclosed manuscript, only a brief summary of the results will be given here.

DFP administered subcutaneously in a single dose ranging from 1-4 mg/kg produced significant alterations in renal function in the unanesthetized rat. Urine volume increased, the osmolality of the urine fell, sodium excretion increased, and the excretion of protein, glucose and blood also increased. Potassium excretion was unaffected. The time course of the response was of such a nature that return to a control status was realized by 24 hours. The primary effects were observed within the first two and a half to six hours after administration of the DFP. These effects were not related to contraction of the bladder, since similar responses were observed in the anesthetized rat where the ureters were cannulated directly. In addition, the volume of urine excreted over a two and a half to six hour period greatly exceeded that that could be held by a normal rat bladder.

these changes were not statistically significant. The only reason for mentioning these effects is to recall that earlier workers (Carter, 1971) demonstrated a similar direction of change in sodium and potassium with the addition of choline esters. Furthermore, in our experiments there was no alteration in total tissue water or inulin space. In addition, the renal slice accumulation of TEA was unaffected. However, both at 2.5 and at 6 hours after the administration of DFP, PAH transport, especially in the presence of lactate, was significantly depressed.

To confirm that the actions of DFP on PAH transport could be a direct effect, a series of in vitro experiments were undertaken. In figure 2 are data which document the necessity of an appropriate period of preincubation of the fresh renal cortex slices with DFP if effects are to be observed. For these experiments, the renal cortex slices were preincubated for the times specified in the figure after which a two hour uptake experiment was performed as in the other experiments to monitor the accumulation of PAH and TEA. It is noteworthy that through 30 minutes of preincubation, DFP had no effect on the transport of PAH or TEA during the subsequent two hour accumulation study. By 60 minutes and thereafter a significant depression of transport occurred. Hence, in all of the subsequent experiments, a preincubation period of 75 minutes was employed before the uptake was measured.

The next three figures (3 through 5) depict time course studies for the effect of DFP on the uptake of PAH and TEA by renal cortex slices. Three concentrations of DFP were studied. The first concentration (5×10^{-6} M) had significant effects on the transport of PAH and TEA, although the bigger effects were observed with PAH. At the higher concentrations of DFP more dramatic effects were seen on PAH and at the highest concentration on TEA. The two hour uptake data are presented as percent of control in figure 6. These dose response data demonstrate that TEA transport is affected in vitro as is the transported of PAH. The accumulation of PAH in the absence of lactate was significantly depressed at all concentrations studied with as large an effect seen at the lowest concentration as at the other two.

These data indicate that DFP can affect directly the accumulation of an organic anion and perhaps an organic cation by renal cortex slices. This effect can be observed under totally in vitro conditions or after the administration of DFP to the intact animal. No alterations in electrolyte distribution were noted nor was tissue oxygen consumption depressed (data not presented), which suggests an effect of DFP other than one of generalized tissue toxicity. The details of mechanisms of the inhibition of organic ion transport are under investigation.

Whatever these mechanisms, it is important to attempt to ascertain whether or not the effects of DFP are mediated through the cholinergic nervous system. One way to test this possibility is to attempt to block the effects of DFP with a cholinergic antagonists such as atropine.

First, the effects of atropine alone were examined. Various concentrations of atropine were added to fresh renal cortex slices and these data are presented in Figure 7. At relatively low concentrations atropine produced a significant reduction in the accumulation of TEA but had no effect on the accumulation of PAH either alone or in the presence of lactate. Given the fact that atropine is a base, these results are not surprising. One might anticipate competitive inhibition of TEA transport by another base.

In Figure 8 our data resulting from experiments in which atropine was administered intraperitoneally sixty minutes before the slice experiment was performed. These data demonstrate clearly that both TEA transport and the transport

of PAH were affected by the atropine. Gosselin and colleagues (Gosselin, et al., 1955; Gosselin, et al., 1960) demonstrated the metabolism of atropine to anionic substances, probably conjugates, and it is probable that it is these metabolites which have interfered with the transport of PAH in this experimental protocol. Once again, TEA transport depression is probably related to a direct competition of the base for transport. Further, since these experiments were performed over a two hour period after pretreatment of the animal with atropine, the data suggests that the effect of atropine administered in vivo is of a relatively long lasting nature as far as renal slice transport is concerned.

In Figure 9 and 10 are data from experiments where DFP and atropine were added together. For the in vivo experiments, a dose of atropine of 5 mg/kg was used which would be expected to interfere with PAH transport. The data in Figure 9 indicate that DFP did have an effect on PAH transport as reported above. However, when atropine was used as a pretreatment and then DFP administered, renal slice transport two and a half hours later was no more depressed than after DFP alone. This might suggest that DFP and atropine are acting through a similar mechanism and that the atropine has produced a depression by itself and blocked a further effect of DFP.

The in vitro experiments are presented in Figure 10 and tend to cloud the issue considerably. Because of the need to preincubate the slices for 75 minutes with DFP in order to see a reasonable effect, the slices were also preincubated with atropine for 75 minutes prior to a 2 hour uptake experiment. Although the variability is considerable in this protocol, there is a tendency for atropine to have an effect in vitro on PAH transport, an effect which was not observed in the earlier experiment where no preincubation was employed. Nonetheless, given that caveat, it would appear that a similar interpretation can be placed on these data as on the in vitro data. That is to say, the effects of the two inhibitors were not additive, but it would appear as if the effect of one is blunted by the effect of the other. These data would be consistent with a common mechanism of action for the atropine and DFP, possibly through a cholinergic mechanism.

At best these data must be viewed as equivocal. Clear-cut inhibition by atropine could not be demonstrated, although the data suggest that atropine or DFP may interfere with the action of the other. The problem is complicated by the fact that both atropine and DFP affect slice transport of organic ions.

Finally, for this report it should be noted that to date no experiments have been conducted with soman. The first grant year commenced on 1 September 1982, renovations in the laboratory were begun promptly, but the delivery of a hood was delayed for approximately 10 weeks. The renovations were completed by mid-March of this year, a final inspection of the laboratory held in June and the first shipment of soman arrived in September 1983.

References

Barajas, L. Innervation of the renal cortex. Fed. Proc. 37: 1192-1201 (1978).

Carter, M.K. Renal electrolyte changes and vasoactive agents. In Renal Pharmacology (J.W. Fisher and E.J. Cafruny, eds.), Appleton-Century-Crafts, New York, 1971, pp. 43-65.

Berndt, W.O. Use of the renal slice technique for the evaluation of renal transport processes. Environ. Health Persp. 15:73-88 (1976).

Berndt, W.O. Methods in Renal Toxicology, In: Methods in Toxicology, edited by A. W. Hayes and R. Dixon, Raven Press, New York (1982).

Kleinzeller, A., Kostyuk, P.G., Kotyk, A. and Lev, A.A. In: Laboratory Techniques in Membrane Physics (H. Passow and R. Stampfli, eds.), Springer-Verlag, New York, 1969, pp. 67-84.

Gosselin, R.E., J.D. Garbourel, S.C. Kalser and J.H. Wills. The metabolism of C^{14} labelled atropine and tropic acid in mice. J. Pharmacol. Exp. Ther. 115: 217-229 (1955).

Gosselin, R.E., J.D. Gabourel and J.H. Wills. The fate of atropine in man. Clin. Pharmacol. Ther. 1: 597-603 (1960).

Figure 1

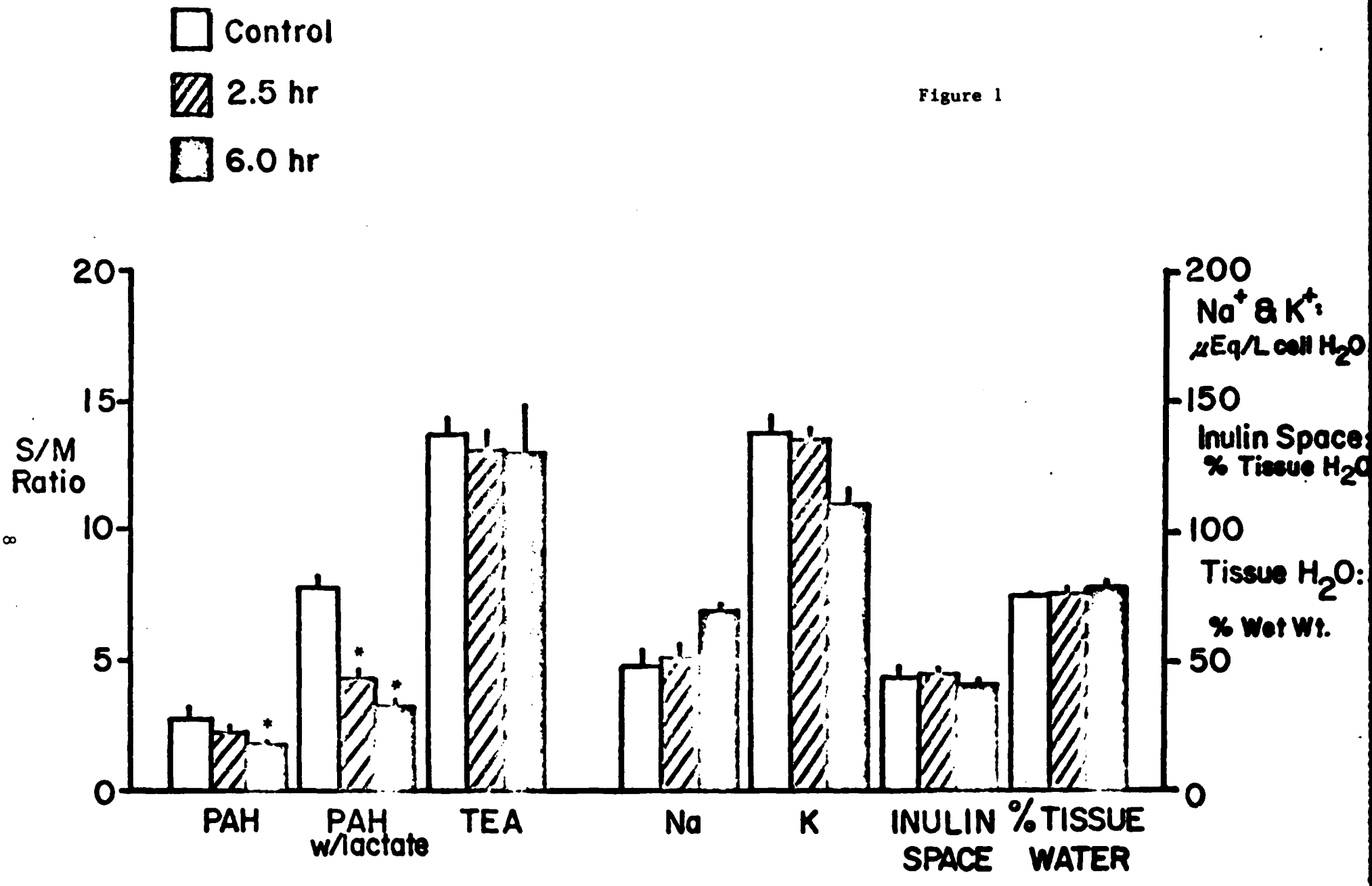

Control
2.5 hr
6.0 hr
S/M Ratio
20
15
10
5
0
200
150
100
50
0
Na+ & K+: µEq/L cell H2O
Inulin Space: % Tissue H2O
Tissue H2O: % Wet Wt.
PAH
PAH w/lactate
TEA
Na
K
INULIN SPACE
% TISSUE WATER

Figure 2

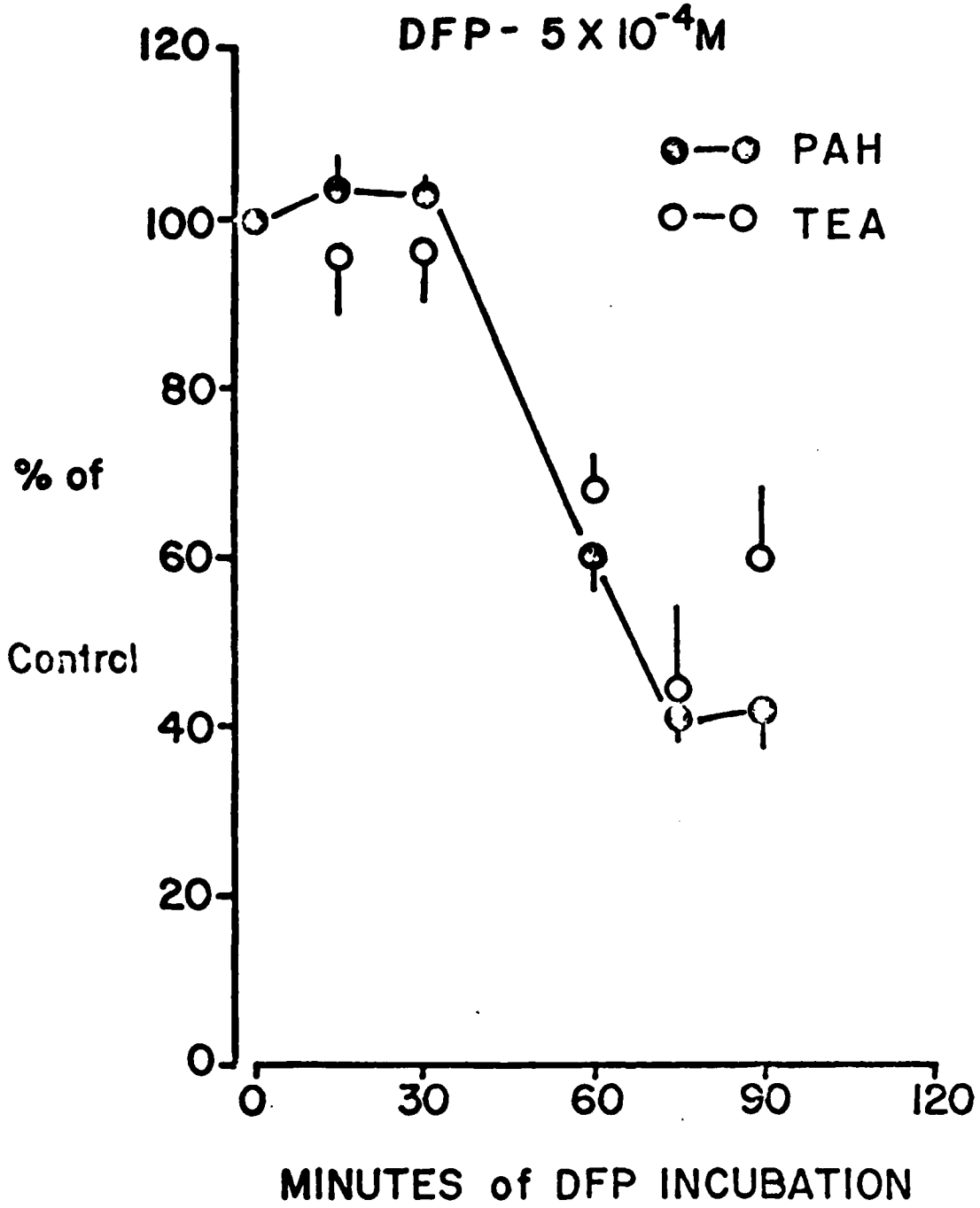
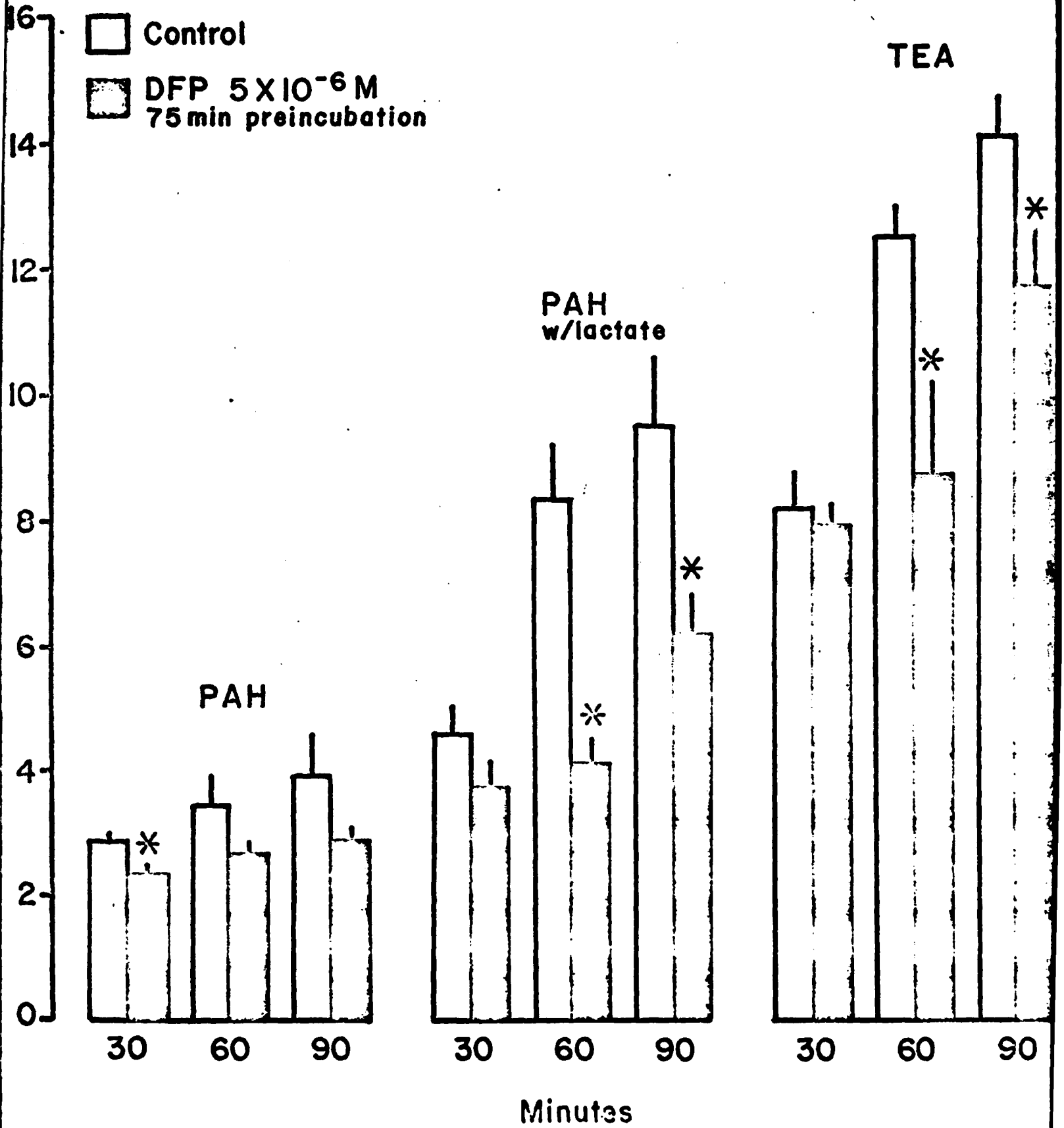

DFP - 5 X 10⁻⁴M
PAH
TEA
120
100
80
60
40
20
0
% of
Control
0
30
60
90
120
MINUTES of DFP INCUBATION

Figure 3


Control
DFP 5X10⁻⁶M
75 min preincubation
TEA
PAH
w/lactate
PAH
16
14
12
10
8
6
4
2
0
30
60
90
30
60
90
30
60
90
Minutes

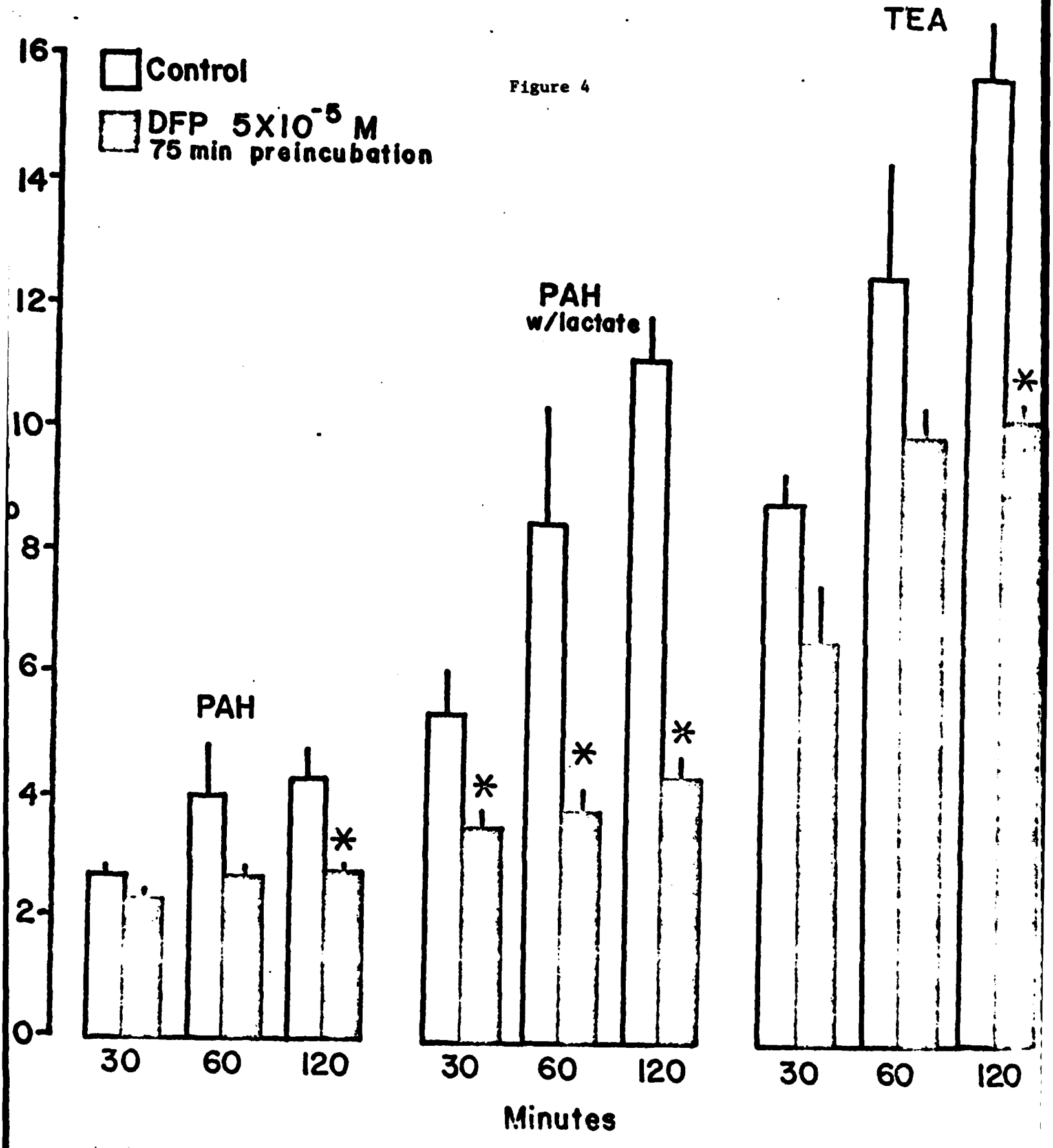

TEA
Control
DFP 5X10⁻⁵ M
75 min preincubation
16
14
12
10
8
6
4
2
0
PAH
PAH
w/lactate
30
60
120
30
60
120
30
60
120
Minutes

Figure 4

Figure 5

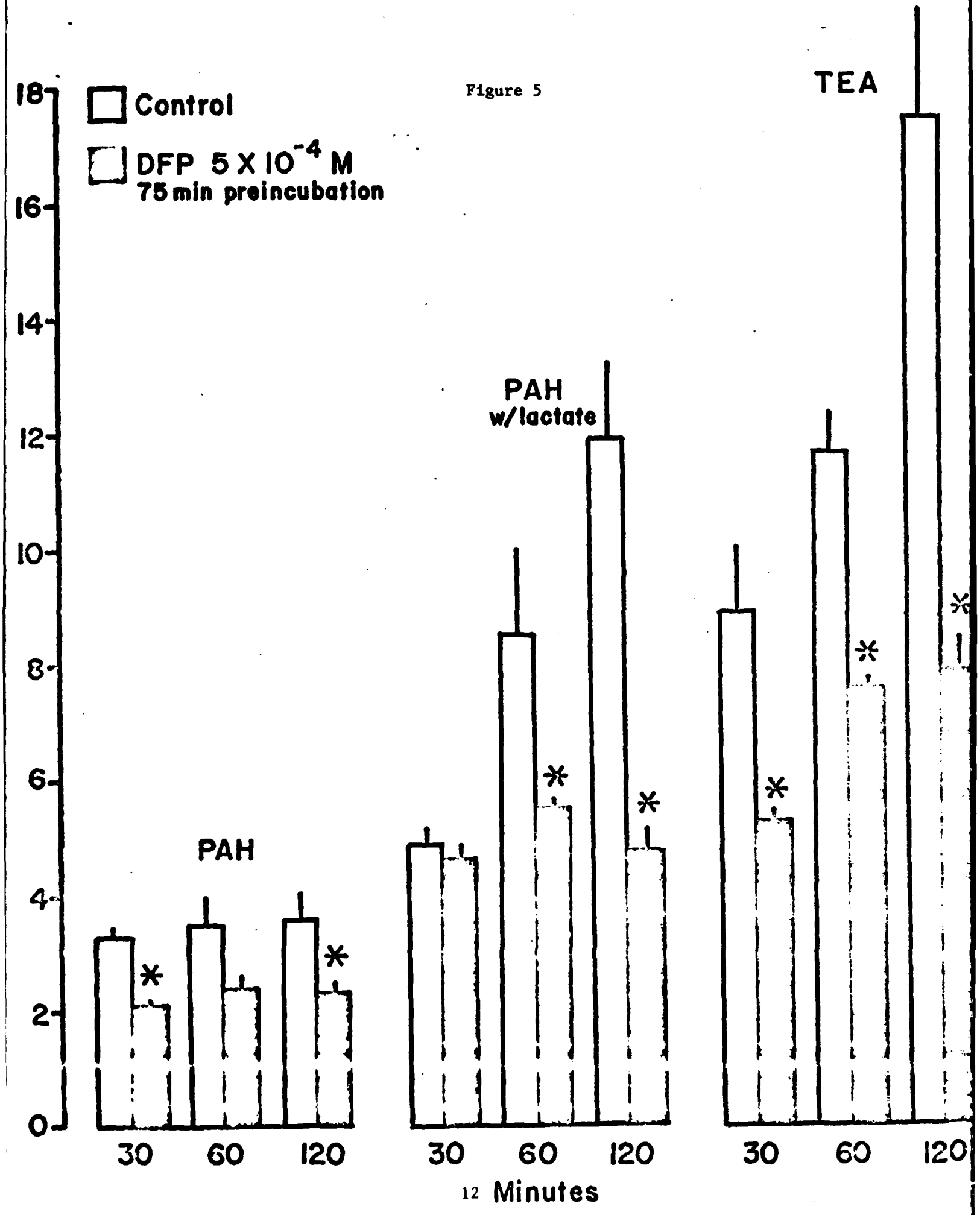

Control
DFP 5 X 10^-4 M
75 min preincubation
PAH
PAH
w/lactate
TEA
0
2
4
6
8
10
12
14
16
18
30
60
120
30
60
120
30
60
120
Minutes

Figure 6

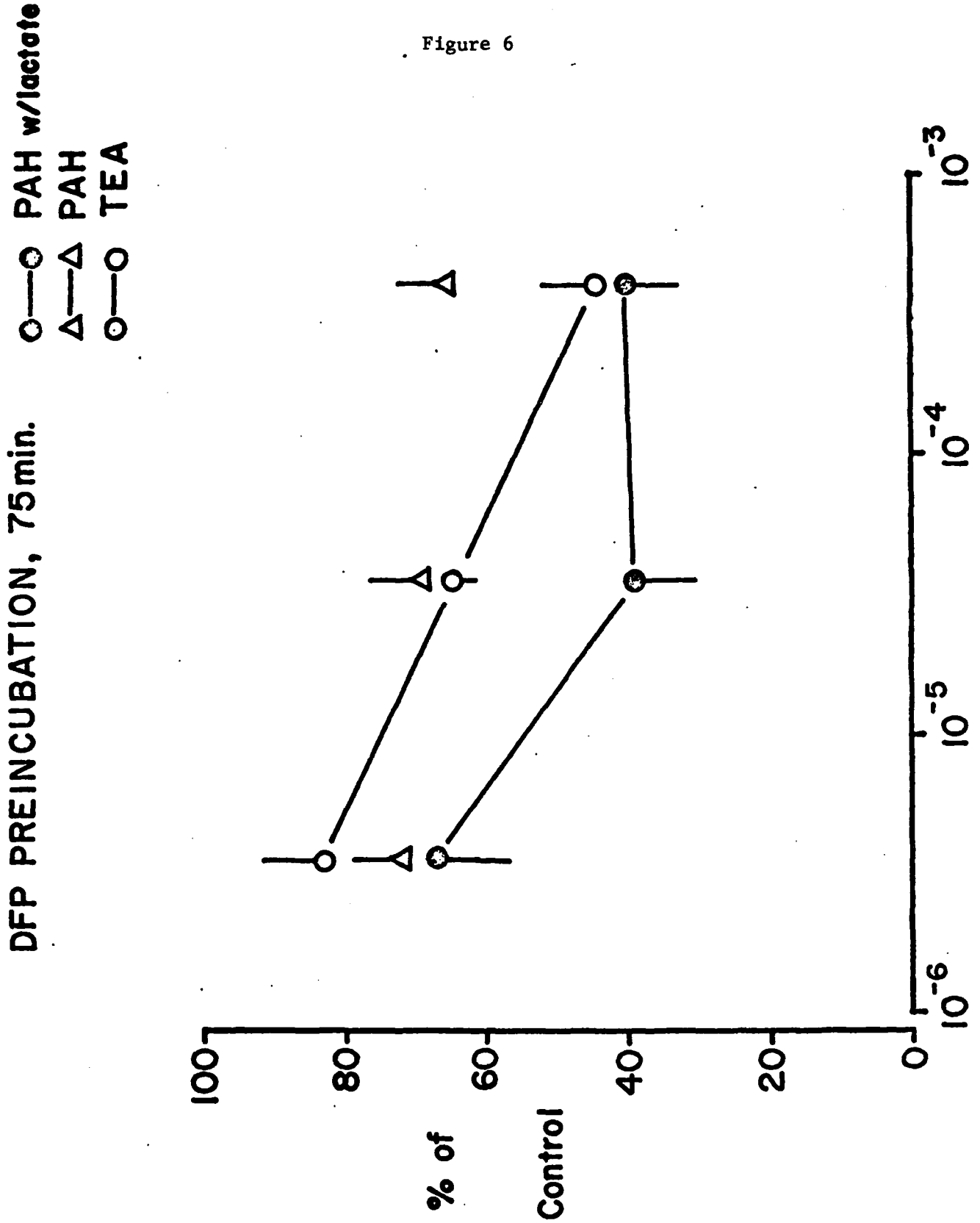

DFP PREINCUBATION, 75min.
O—O PAH w/lactate
Δ—Δ PAH
O—O TEA
% of Control
100
80
60
40
20
0
10-6
10-5
10-4
10-3
DFP, M

Figure 7

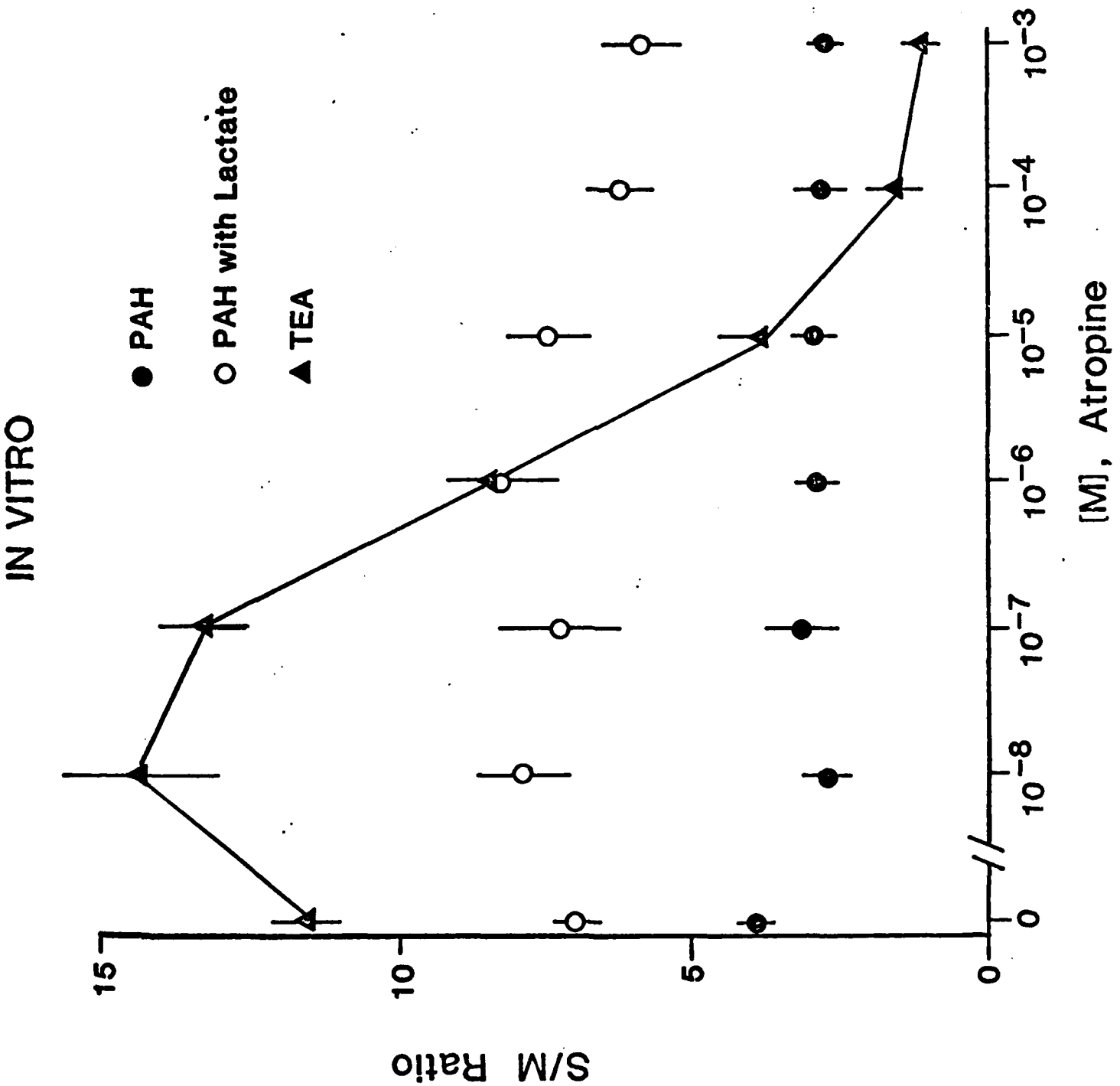

IN VITRO
● PAH
○ PAH with Lactate
▲ TEA
S/M Ratio
15
10
5
0
0
10⁻⁸
10⁻⁷
10⁻⁶
10⁻⁵
10⁻⁴
10⁻³
[M], Atropine

Figure 8

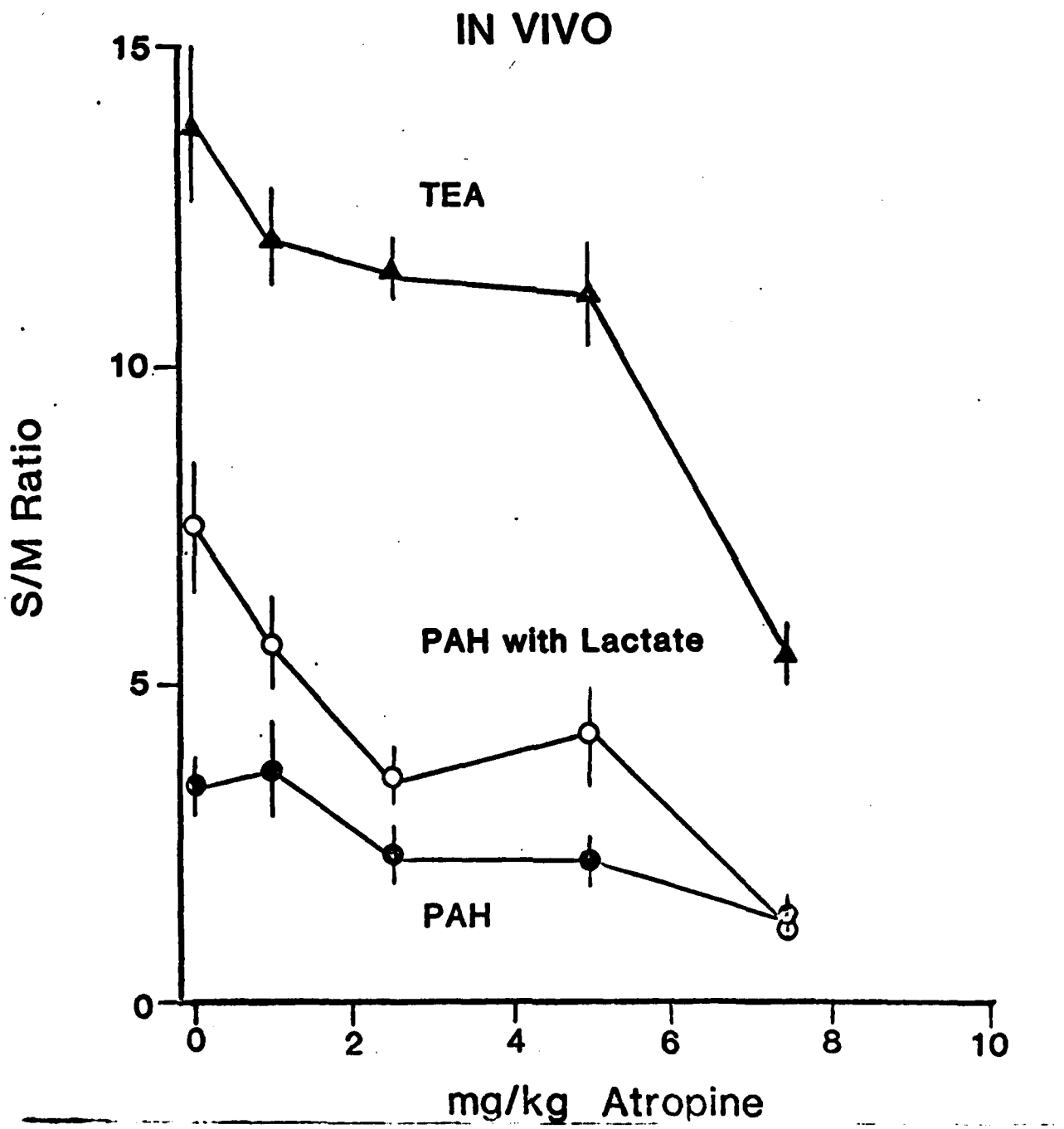

IN VIVO
15
10
5
0
S/M Ratio
TEA
PAH with Lactate
PAH
0
2
4
6
8
10
mg/kg Atropine

Figure 9

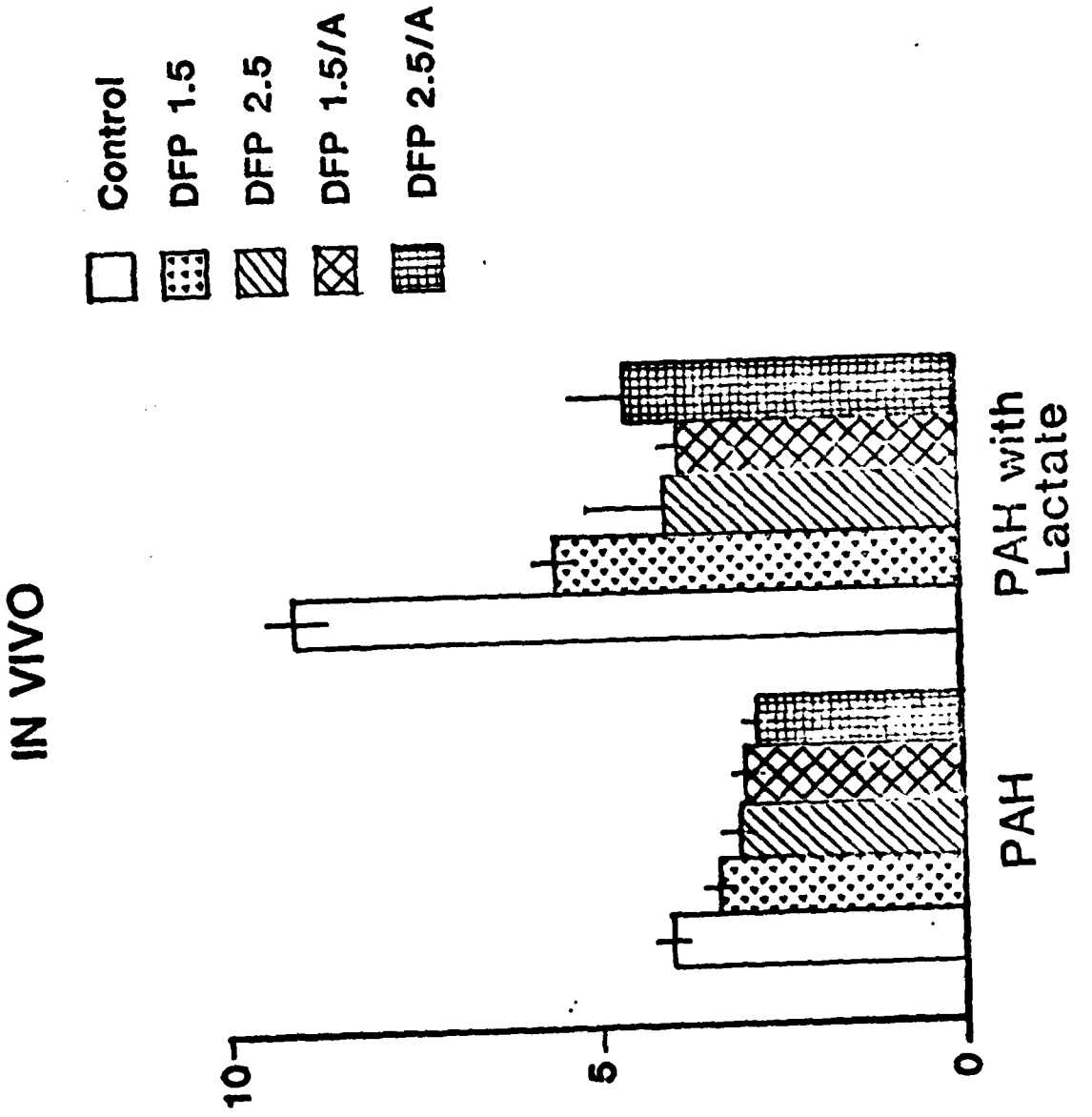
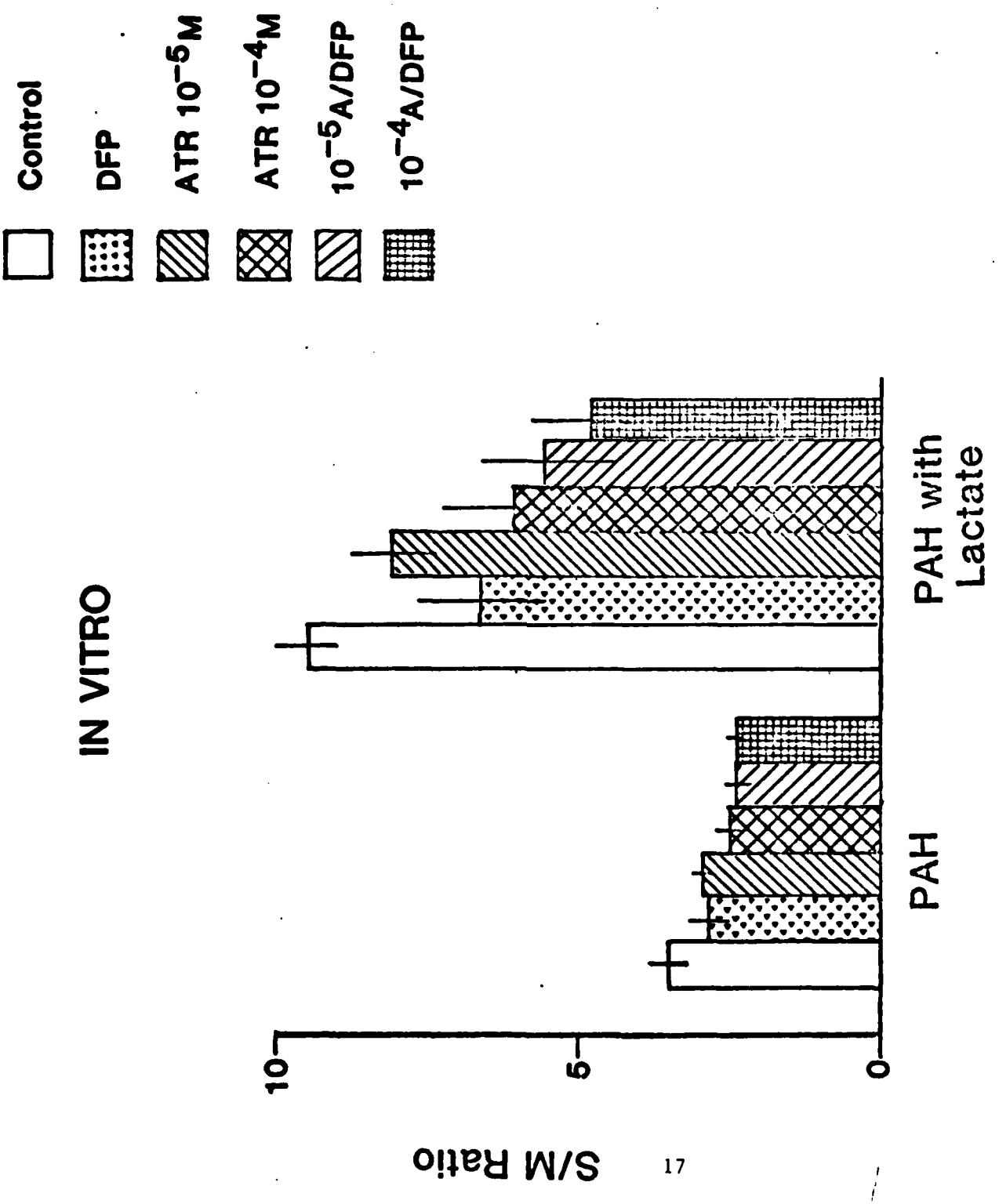

IN VIVO
Control
DFP 1.5
DFP 2.5
DFP 1.5/A
DFP 2.5/A
10
5
0
PAH
PAH with Lactate

Figure 10


Control
DFP
ATR 10^{-5}M
ATR 10^{-4}M
10^{-5}A/DFP
10^{-4}A/DFP
IN VITRO
PAH with Lactate
PAH
S/M Ratio
10
5
0

17

APPENDIX

Effects of diisopropylfluorophosphate (DFP) on renal function in the rat.

W. O. Berndt, J. Baggett, B. Hoskins, D. K. Lim, and I. K. Ho

Department of Pharmacology
College of Medicine
University of Nebraska Medical Center
Omaha, Nebraska, USA

and

Department of Pharmacology and Toxicology
University of Mississippi Medical Center
Jackson, Mississippi, USA

Editorial Correspondence and Reprint Requests to:

Dr. W. O. Berndt
Department of Pharmacology
College of Medicine
University of Nebraska Medical Center
42nd and Dewey Avenue
Omaha, Nebraska 68105
USA

KEY WORDS

DFP
Cholinesterase inhibitors
DFP and renal function
DFP toxicity
Cholinesterase inhibitors and renal function
DFP and sodium excretion

SUMMARY

Numerous studies have suggested a role for the nervous system in renal function. Several cholinergic agents have been examined for effects in vivo and in renal slices. Effects mediated through the vascular system and also direct effects have been reported. In this study the irreversible cholinesterase inhibitor, diisopropylfluorophosphate (DFP) in doses of 1-4 mg/kg, was tested on renal function. A single dose of DFP (2, 3 or 4 mg/kg) caused an increased flow of urine of low osmolality over the 6 hours after the administration of the drug with essentially return to control status by 24 hr after either of the lower doses. Twenty-four hours after 4 mg/kg, urine volume was less and urine osmolality greater than control. Renal and brain cholinesterases remained depressed 24 hours after DFP. Inulin clearance was increased by the lower doses and decreased by the highest dose in anesthetized rats. Renal blood flow measured with an electromagnetic flow meter showed a similar dose-response relationship. However, urine flow increased at all doses. The increased urine flow associated with decreased inulin clearance (4 mg/kg) and renal blood flow (3 or 4 mg/kg) suggest a direct effect of DFP on renal tubular function. These effects do not appear to be related to inhibition of cholinesterase.

INTRODUCTION

The clearest evidence to suggest a role for the autonomic nervous system in the regulation of renal function may well have come from the studies of Barajas and colleagues (e.g., 2, 4, 5, 6, 7, 22; reviewed by Barajas, 3). These studies, mostly morphological and histochemical, have yielded evidence of an extremely rich adrenergic innervation of the kidney in most species and an interesting relationship between acetylcholinesterase and that adrenergic innervation. Several investigators (16, 24, 28, 29; reviewed by DiBona, 15) have presented physiological data that suggest a direct role of the sympathetic nerves in renal tubular sodium reabsorption, i.e., not mediated through alterations of glomerular filtration rate (GFR) or renal blood flow (RBF).

Direct effects of cholinergic agents on renal function and transport appear to be more complicated, however. Earlier work by Carter, Vander and others (26, 27, 23, 19, 21, 12) demonstrated that there were effects of cholinergic agents, but Earley and Friedler (17) suggested that the increase in urine flow due to acetylcholine, for example, was due to an increase in medullary blood flow, thus resulting in a washout of the medullary interstitium. Hence, these early studies suggested that whatever effects were exerted on renal function by cholinergic agents were related to increases in renal blood flow because cholinergic agents are vasodilators. The work of Carriere et al. (10) called this interpretation into question, since these workers found no change in medullary blood flow after administration of acetylcholine or other vasodilators. These studies utilized the krypton washout technique. Further Avrunin and Carter (1) found no washout of the cortico-medullary sodium gradient in the rat kidneys after administration of bethanechol.

The possiblity of direct effects of cholinomimetics on renal function was investigated by Carter and colleagues (12, 13, 14, 11). Through these many investigations, Carter's group demonstrated that acetylcholine and propionylcholine could alter renal slice function after direct addition of these compounds. When used in high concentrations, increases in tissue sodium and decreases in tissue potassium were observed. Tissue oxygen consumption was increased. Atropine blocked the effects of acetylcholine on sodium accumulation in these slice studies. Although it is by no means clear how these data fit into the overall scheme of a cholinomimetic-induced diuresis, they do demonstrate a direct action of these compounds on renal cell electrolyte balance.

Carter's group did not examine the effects of cholinesterase inhibitors on overall renal function or on renal slice electrolyte balance. Cholinesterase inhibitors might have effects on renal function similar to those of cholinomimetics, or might act through direct mechanisms related to the inherent reactivity of these active phosphorylating agents. The present study was undertaken to examine the effects of organophosphate cholinesterase inhibitors, specifically diisopropylfluorophosphate (DFP), on renal function in the rat.

MATERIALS AND METHODS

Male Sprague-Dawley rats (approximately 200-250 g) from Charles River Laboratories, Wilmington, MA or Blue Spruce Farms, Altamont, NY were used in these studies. DFP was purchased from Calbiochem. Comparable effects were obtained with each of the two lots used (#101318 and #201781). Single doses of DFP were administered subcutaneously as indicated. Prior to and during the experiments, the animals were housed in a room with automatic 12 hour light-dark cycles and a controlled temperature of 25°C.

For the experiments with unanesthetized animals, the rats were housed individually in stainless steel metabolism cages. One 24-hr period of adaptation was allowed before any urine collections. Subsequently two or three control days were allowed before injection of the DFP subcutaneously. On each control and experimental day, food was not available during the first six hours (0830-1430 hours), although water was available ad libitum. Powdered Purina Rat Chow was provided during the remaining 18 hours of each control or experimental day. Three urine samples were collected on each day: 0-2.5 (0830-1100 hours) hours, 2.5-6.0 hours and over the last 18 hours of each day. Water and food consumption were determined for each 24 hour period, and the animals were weighed daily.

Urine samples were analyzed for volume, osmolality, sodium, and potassium. Excretion of glucose, protein and blood were assessed qualitatively with BiliLabStix (Ames).

Cholinesterase activity was assessed by the method of Ellman et al. (18). Kidneys and brains were homogenized in 0.1 M phosphate buffer, pH 8.0, in a Potter-Elvehjem homogenizer with approximately 20-40 mg of tissue per ml. Aliquots (no more than 0.4 ml) of the homogenate were added to cuvettes containing 2.6 ml of 0.1 M phosphate buffer and 5,5'-dithiobis-2-

nitrobenzoic acid (DTNB) reagent (0.01 M in 0.1 M phosphate buffer, pH 7.0). Twenty microliters of acetylthiocholine iodide (0.075 M) or butyrylthiocholine iodide (0.075 M) were added to the sample cuvette. The absorbance was measured at 412 nm with a Beckman-2600K spectrophotometer or a Gilson spectrophotometer and changes in absorbance were recorded. In studies of the in vitro inhibitory effects of DFP, the same procedures were used except that various concentrations of DFP were added directly to the reaction mixture.

In the experiments where hemodynamic parameters were measured the rats were anesthetized with pentobarbital (60 mg/kg i.p.). Inulin clearance was measured to assess glomerular filtration rate (GFR), and total renal blood flow was monitored with an electromagnetic flow meter (Carolina Medical Electronics, Inc.). One jugular vein and one carotid artery were cannulated with polyethylene tubing (PE 50), and both ureters were cannulated with PE 10 tubing. A tracheal cannula also was inserted to facilitate breathing. A saline solution (0.15 M sodium chloride) containing ^{14}C-inulin was infused i.v. at 0.3 ml/min for 15 minutes and subsequently at 0.075 ml/min. Blood samples were taken periodically over the course of the experiment. Urine was collected at 15 minute intervals with two control urine samples collected before the subcutaneous injection of the DFP. DFP was administered in doses of 1 mg/kg, SQ, at one-half hour intervals. Inulin in urine and plasma was assayed by liquid scintillation spectrometry. Although blood flow was measured continuously, the values at the mid-point of each urine collection were used for statistical analyses and data presentation. The two values for control and each dose of DFP were averaged, as were the urine flows and inulin clearances.

Urine osmolality was measured by freezing point depression with an Advanced Laboratory Osmometer, Model 3L. Urinary sodium and potassium

concentrations were assessed with flame emission photometry (Instrumentation Laboratories, Model 343) utilizing lithium as an internal standard.

Statistical analyses were performed with Student's t test or an Analysis of Variance with the significance of the differences between means determined by Student-Neuman-Keuls test (25). Probability values of $p < .05$ were accepted as significant.

RESULTS

The data in Figure 1 are renal cholinesterase measurements which correspond in time to the three urine collections made in the experiments with unanesthetized animals. In Panel A are data obtained with kidney and brain utilizing acetylthiocholine as substrate. In Panel B are similar data with butyrylthiocholine as substrate to monitor non-specific esterases. Of the three time periods measured, maximal effects were observed at 2.5 hours for kidney. Significant inhibition of enzyme activities persisted at 24 hours after the single dose of DFP, although there was a tendency for the renal enzyme activity to recover. Recovery of brain cholinesterase over this time period was less marked than with kidney.

Experiments with unanesthetized animals. Body weight changes, food and water consumption data have been published elsewhere by Lim et al. (20). For completeness, these are summarized here. Only the two higher doses of DFP (3 or 4 mg/kg) altered body weight, food or water consumption. A 10-15% decrease in body weight was observed by 24 to 48 hours after DFP 4 mg/kg. A lesser effect was noted with 3 mg/kg and no effect at the lower doses. Normal rates of body weight gain were present for each group by 3 days after DFP. Food consumption was decreased to 60% of control by the second day after the largest dose of DFP, and all groups of animals had normal food consumption by day 3 after DFP. A modest, but significant, increase in food consumption was observed with the high dose group from days 5 through 8. Water intake fell 50% from control levels of approximately 15 ml/100 g/day within 24 hours of DFP administration with recovery by 48 hours.

Urinary excretion of glucose, blood and protein (mostly albumin) also

occurred with the higher doses of DFP. The data in Figure 2 depict results with 3 mg/kg, but similar results were seen with 4 mg/kg. These semi-quantitative assessments indicated that major urinary glucose excretion occurred earlier than urinary excretion of other substances. Gross protein excretion occurred late. Significant quantities of blood appeared throughout the 30 hours following DFP administration with peak excretion seen between 2.5 and 6 hours.

The data presented in figures 3 through 6 are for the first six hours of each control or experimental day. The urine flow increased significantly after each dose of DFP as compared to each group of animals' control urine or compared to the urine flow of a control, untreated group (Figure 3). As the urine flow increased, the urinary osmolality fell significantly (Figure 4). The urinary sodium excretion increased significantly over the first six hours of the post-treatment period (Figure 5). Urinary potassium excretion over the same time period was not changed significantly (Figure 6).

The urinary flow rates computed for each of the three time periods are presented in Figure 7. At each dose of DFP, the maximal response was seen in the first 2.5 hours. Indeed the majority of the effect seen over the first six hours post-treatment (Figure 3) was attributable to the early effect. At the highest dose of DFP, a significant depression of urine flow was observed over the last 18 hours post-treatment. Changes in urinary osmolalities were consistent with the changes in volume (Figure 8). Maximal decreases in osmolality occurred when the urinary flow rate was the highest at each dose of DFP. The osmolality of the urine collected 24 hours post-treatment was significantly elevated after the two higher doses of DFP.

Urinary electrolyte excretion over the last 18 hours of each

experiment is presented in Figure 9. Although DFP did not affect potassium excretion over the first six hours of the experiment, significant decreases were noted from 6-24 hours post-treatment with the two higher doses of DFP. Urinary sodium excretion also was decreased significantly after the highest dose of DFP.

Experiments with anesthetized animals. The data in Figure 10 represent blood flows, inulin clearances (GFR), and urine flows for control and each of four DFP treatments. Although urine flow showed a consistent increase with each subsequent dose of DFP, neither blood flow nor GFR showed consistent changes. The most striking dissociation of urine flow from hemodynamic parameters was observed in the last period (total dose of 4 mg DFP/kg) where both blood flow and inulin clearance fell markedly while urine flow remained elevated.

DISCUSSION

The renal response to an acute dose of DFP had a short latency and was not sustained. The increased excretion of water and sodium is consistent with the earlier observations of Carter and colleagues (12, 23, 21), and of Vander (26, 27). In these studies the direct effects of cholinomimetic agents were investigated. Although these effects may have been related to an increased blood flow which resulted from vasodilation, experimental data to support this supposition were not forthcoming (10). Further, the data presented here also do not support that suggestion unequivocally. Although total renal blood flow increased significantly at low doses of DFP, this effect was not sustained at higher doses. Furthermore, regardless of the status of blood flow or GFR, urine flow rose, suggestive of a tubular action of DFP. Similar experiments were performed with neostigmine (data not presented). Although neostigmine has a much shorter duration of action than DFP no effects on urine flow or GFR were observed. Renal blood flow fell toward the end of each experiment, but at no time in any experiment was blood flow increased. At the mid-point of the urine collection periods when blood flow was measured renal acetylcholinesterase ranged from 14 to 30% of control depending on the dose of neostigmine. These observations further substantiate the suggestion that the effects of DFP were not related to inhibition of cholinesterase and may represent a direct effect of this reactive compound.

Finally, it is noteworthy that after DFP renal cholinesterase activities, unlike renal function, failed to return to normal, although the tendency for the renal enzyme activities to recover was greater than that of brain. That is, a significant reduction in renal enzyme activity persisted even at 24 hours after administration of a single dose of DFP

when renal function parameters had returned to normal. In addition, Carter (12) noted that whenever a cholinomimetic enhanced sodium excretion, potassium excretion increased as well. This was not true in these studies. The control urinary potassium concentrations, however, varied considerably from day to day, thereby perhaps obscuring any effects of DFP. That is, although DFP tended to increase potassium excretion (note particularly trends with 2 or 4 mg DFP/kg, variability of controls obviated any significance being attributed to these effects. Note that the control values in the 3 mg/kg protocol varied by almost 100%. While the DFP effect was less even when compared to a single, minimum control value. Although sodium excretory controls may have varied as much as those for potassium, the DFP effect was from 2.5 to 4.5 times control.

It is interesting to note that the enhanced sodium excretion over the first 6 hours was compensated for fully by a reduced sodium excretion from 6 to 24 hr. The significant decrease in potassium excretion over this 18 hr. period remains unexplained since a significant increase in potassium excretion did not occur early in the experiment. The decreased osmolality observed after DFP was consistent with the increased urine flow over the same time.

Because the action of DFP on renal function was relatively short-lived, it might be suggested that the increase in urine flow was due to cholinergic stimulation of bladder contraction. This is not likely for two reasons. First, the volumes of urine excreted over the first 6 hours were considerably larger than could be contained in the rat urinary bladder. Secondly, in the experiments with anesthetized rats the ureters were cannulated directly, obviating any urinary excretory effect mediated through bladder contraction.

Whether or not this effect of DFP reflects a nephrotoxic response or a

diuretic effect is unclear. Semi-quantitative assessment of urinary protein and glucose excretion were undertaken, but the data were not absolutely unequivocal. At the highest dose of DFP studied (4 mg/kg), urinary glucose excretion increased many fold from less than 1 μg/hr to over 30, with a lesser response at lower doses (see Fig. 2). Increased urinary glucose excretion might well be indicative of a nephrotoxic response (8, 9), although no effect was observed on glucose excretion at the lowest dose of DFP despite alterations in other renal function parameters. Increased urinary protein excretion also was observed at the higher doses, but did not coincide with the increased glucose excretion. Although the urinary excretion of blood might suggest renal damage, it also might be indicative of damage to the ureters or urinary bladder. Overall, however, a contemporaneous increase in the urinary excretion of glucose, blood and protein certainly suggests the occurrence of acute renal damage.

Whatever the effect of DFP on renal function in the rat, the action appears to be unrelated to the well known cholinergic effects of this organophosphate cholinesterase inhibitor. However, the chemical reactivity of DFP may be responsible for the effects observed. That is, the DFP may react with important renal tissue sites involved with normal homeostatic renal mechanisms and through this interaction alter renal function or produce a modest, self-limiting acute renal damage. Studies are presently underway to examine possible direct effects of DFP on specific renal transport mechanisms in rat renal tissue. Direct effects on PAH transport, for example, might be indicative of the reaction of DFP with the renal PAH transport site.

ACKNOWLEDGEMENTS

This work was supported by Department of the Army grants DAMR 17-82-C-2220 and DAMR 17-81-C-1238. The authors are indebted to Bonnie Berndt, Connie Andersen and Ken Johnson for their excellent technical assistance.

REFERENCES

1. B. Avrunin and M.K. Carter, Effects of bethanechol (Urecholine) on renal electrolyte excretion in rats and chickens. Proc. Soc. Exp. Biol. Med., 133 (1970) 901-907.

2. L. Barajas, The ultrastructure of the juxtaglomerular apparatus as disclosed by three dimensional reconstructions from serial sections. The anatomical relationship between the tubular and vascular components. J. Ultrastruct. Res., 33 (1970) 116-147.

3. L. Barajas, Innervation of the renal cortex. Fed. Proc., 37 (1978) 1192-1201.

4. L. Barajas and J. Muller, The innervation of the juxtaglomerular apparatus and surrounding tubules: A quantitative analysis by serial section electron microscopy. J. Ultrastruct. Res., 43 (1973) 107-132.

5. L. Barajas, A.J. Silverman and J. Muller, Ultrastructure localization of acetylcholinesterase in the renal nerves. J. Ultrastruct. Res., 49 (1974) 297-311.

6. L. Barajas and P. Wang, Demonstration of acetylcholinesterase in the adrenergic nerves of the renal glomerular arterioles. J. Ultrastruct. Res., 53 (1975) 244-253.

7. L. Barajas, P. Wang and S. DeSantis, Light and electron microscopic localization of acetylcholinesterase activity in the rat renal nerves. Amer. J. Anat., 147 (1976) 219-233.

8. W. O. Berndt, Renal function tests: What do they mean? A review of renal anatomy, physiology and biochemistry. Environ. Health Persp., 15 (1976) 55-71.

9. W. O. Berndt, Methods in renal toxicology, In: Methods in Toxicology, edited by A. W. Hayes and R. Dixon, Raven Press, New York, 1982, pp. 447-474.

10. S. Carriere, J. Friborg and J. P. Guay, Vasodilators, intrarenal blood flow, and natriuresis in the dog. Amer. J. Physiol., 221 (1971) 92-98.

11. M.K. Carter, Effects of choline esters and ouabain on sodium and potassium content of incubated rat kidney cortex slices. Fed. Proc. 28 (1969) 547.

12. M.K. Carter, Renal electrolyte changes and vascactive agents. In Renal Pharmacology (J. W. Fisher and E. J. Cafruny, eds.), Appleton-Century-Crafts, New York, 1971, pp. 43-65.

13. M.K. Carter and M.E. Greig, Relationship between ion transport and cholinesterase activity in kidney cortex slices from normal and adrenalectomized rats. Fed. Proc., 14 (1955) 324-325.

14. M.K. Carter, G.R. Hemstreet and J.W. Reid, Effects of pilocarpine on sodium and potassium transport in kidneys of intact rats and in kidney slices. The Pharmacologist, 3 (1961) 57.

15. G.F. DiBona, Neurogenic regulation of renal tubular sodium reabsorption. Amer. J. Physiol., 233 (1977) F73-81.

16. G.F. DiBona, E.J. Zambraski, A.J. Aquilera and G.J. Kaloyanides, Neurogenic control of renal tubular sodium reabsorption in the dog. Circulation Res., 40(suppl. I) (1977) I-127 - I-130.

17. L.E. Earley and R.M. Friedler, Studies on the mechanism of naturesis accompanying increased renal blood flow and its role in the renal response to extracellular volume expansion. J. Clin. Invest., 44 (1965) 1857-1867.

18. G.L. Ellman, K.D. Courtney, V. Andres Jr. and R.M. Featherstone, A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7 (1961) 88-94.

19. J.P. Hayslett, M. Kashgarian and F.H. Epstein, The diuresis of renal

vasodilation. Clin. Res., 16 (1968) 385.

20. D.K. Lim, B. Hoskins and I.K. Ho, Assessment of diisopropylfluorophosphate (DFP) toxicity and tolerance in rats. Res. Comm. Chem. Pathol. Pharmacol., (1983) in press.

21. D.G. May and M.K. Carter, The effects of vasoactive agents on urine and electrolyte excretion in the chicken. Amer. J. Physiol., 218 (1970) 417-422.

22. J. Muller and L. Barajas, Electron microscopic and histochemical evidence for a tubular innervation in the renal cortex of the monkey. J. Ultrastruct. Res., 41 (1968) 533-549.

23. M.L. Parmalee and M.K. Carter, The diuretic effect of acetylcholine in the chicken. Arch. Int. Pharmacodyn., 174 (1968) 108-113.

24. H.W. Schrier, Effects of adrenergic nervous system and catecholamines on systemic and renal hemodynamics, sodium and water excretion and renal secretion. Kidney Intern., 6 (1974) 291-306.

25. R.R. Sokal and E.J. Rohlf, Biometry, W.H. Freemont Company, San Francisco (1969).

26. A.J. Vander, Effects of acetylcholine, atropine and physostigmine on renal function of the dog. Amer. J. Physiol., 206 (1966) 492-504.

27. A.J. Vander, Direct effects of prostaglandins on renal function and renin release in anesthetized dogs. Amer. J. Physiol., 214 (1968) 218-230.

28. E.J. Zambraski, G.E. DiBone and G.J. Kaloyanides, Effect of sympathetic blocking agents on the antinaturesis of reflex renal nerve stimulation. J. Pharmacol. Exptl. Therap., 198 (1976a) 464-472.

29. E.J. Zambraski, G.E. DiBona and G.J. Kaloyanides, Specificity of neural effect on renal tubular sodium reabsorption. Proc. Soc. Exptl. Biol. Med., 151 (1976b) 543-546.

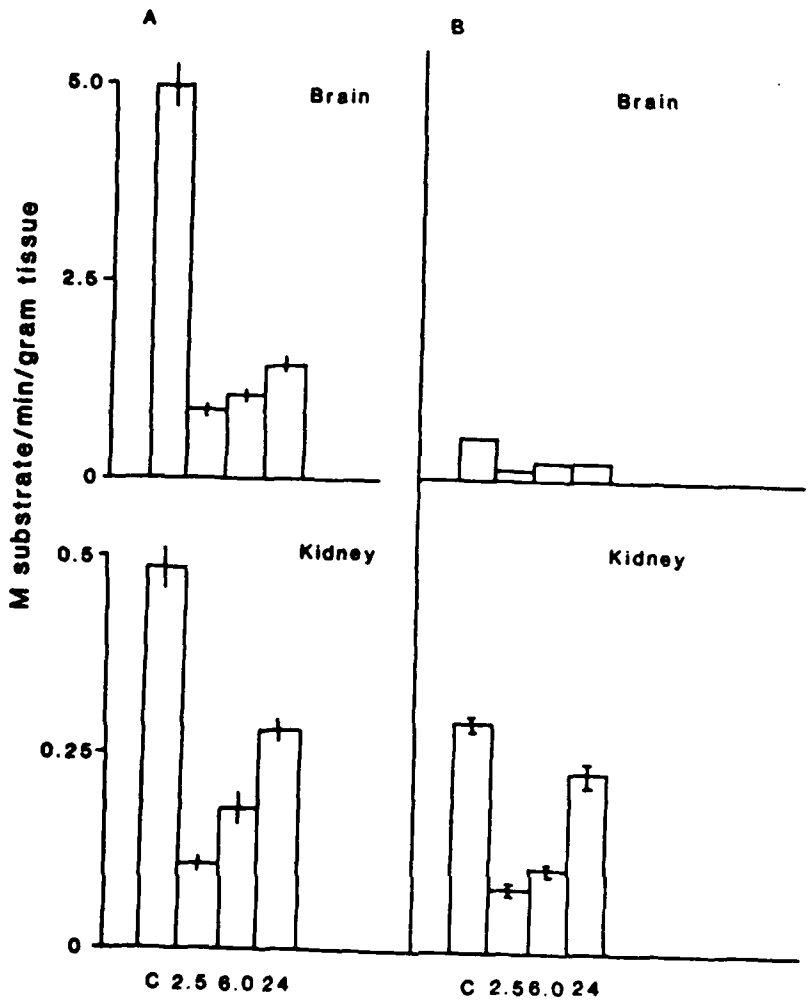


Figure 1. Renal and brain cholinesterase activity measured at various times after DFP (2 mg/kg). The numbers at the bottom of the bars refer to hours after DFP administration and C represents control. The height of the bar is the mean and the vertical line the standard error for N = 6.

Panel A. Acetylthiocholine as substrate.

Panel B. Butyrylthiocholine as substrate.

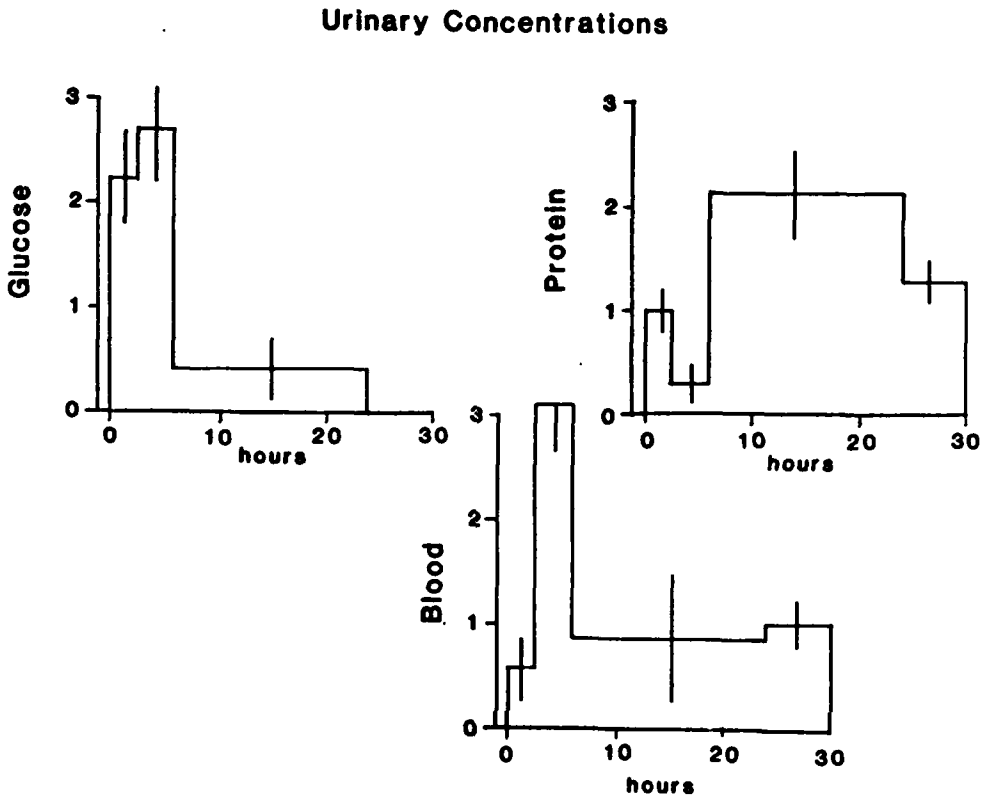


Figure 2. Urinary concentration of glucose, blood or protein after a single dose of DFP (3 mg/kg). The height of the bar is the mean and vertical line the standard error for N = 6. The vertical axis represent arbitrary values for each substance measured.

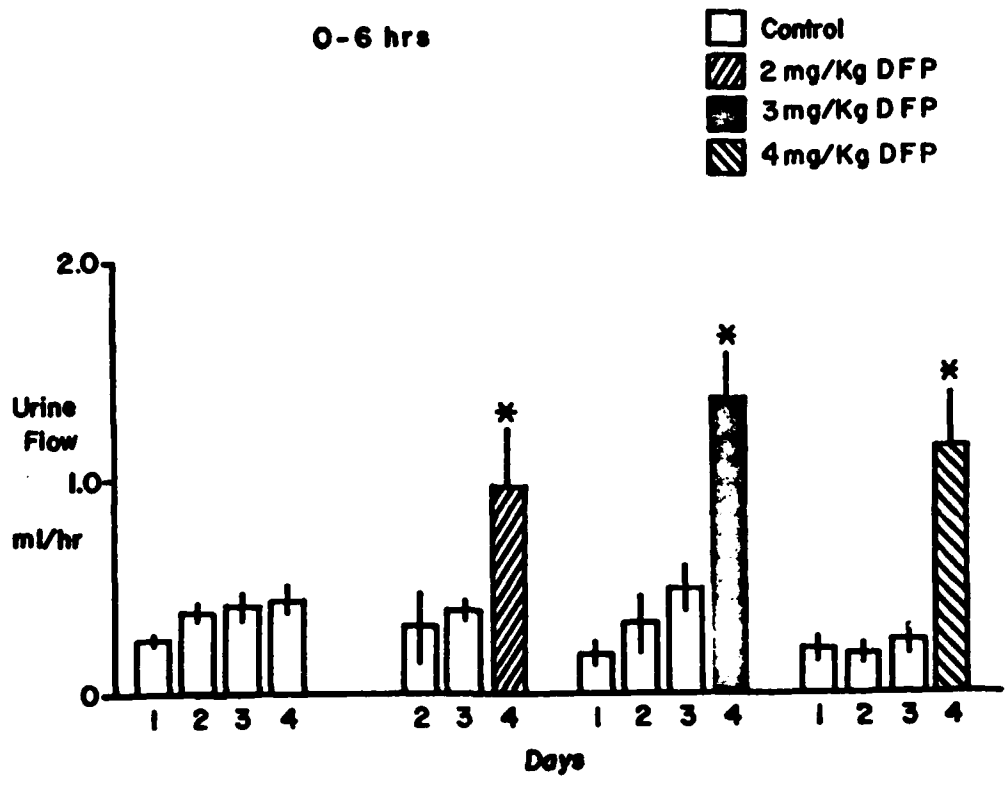


Figure 3. Effect of DFP on six hour urine flow. The unfilled bars represent controls and the filled bars the six-hour urine flow at each dose of DFP. The height of each bar is the mean and the vertical line the standard error for N = 4. Statistically significant values ($p < .05$) are indicated by asterisks.

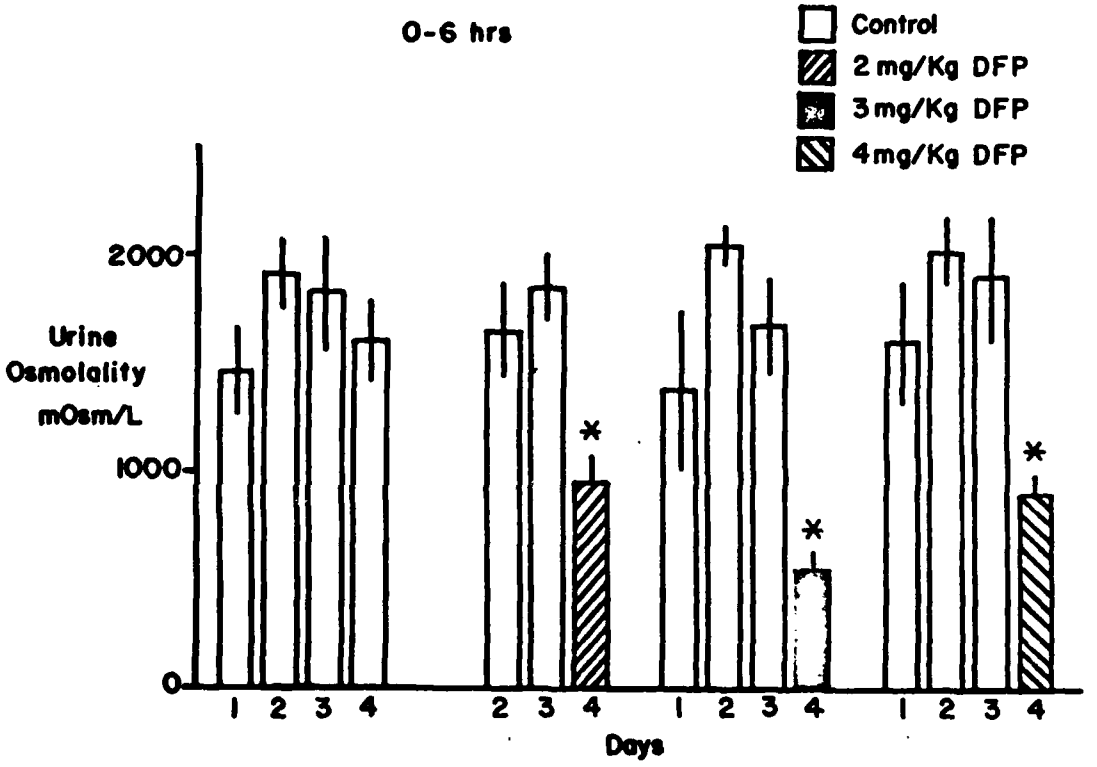


Figure 4. Effect of DFP on urine osmolality. The unfilled bars represent controls and the filled bars the osmolality of the urine collected for 6 hours after the indicated dose of DFP. The height of each bar is the mean and the vertical line the standard error for N = 4. Statistically significant values ($p < .05$) are indicated by asterisks.

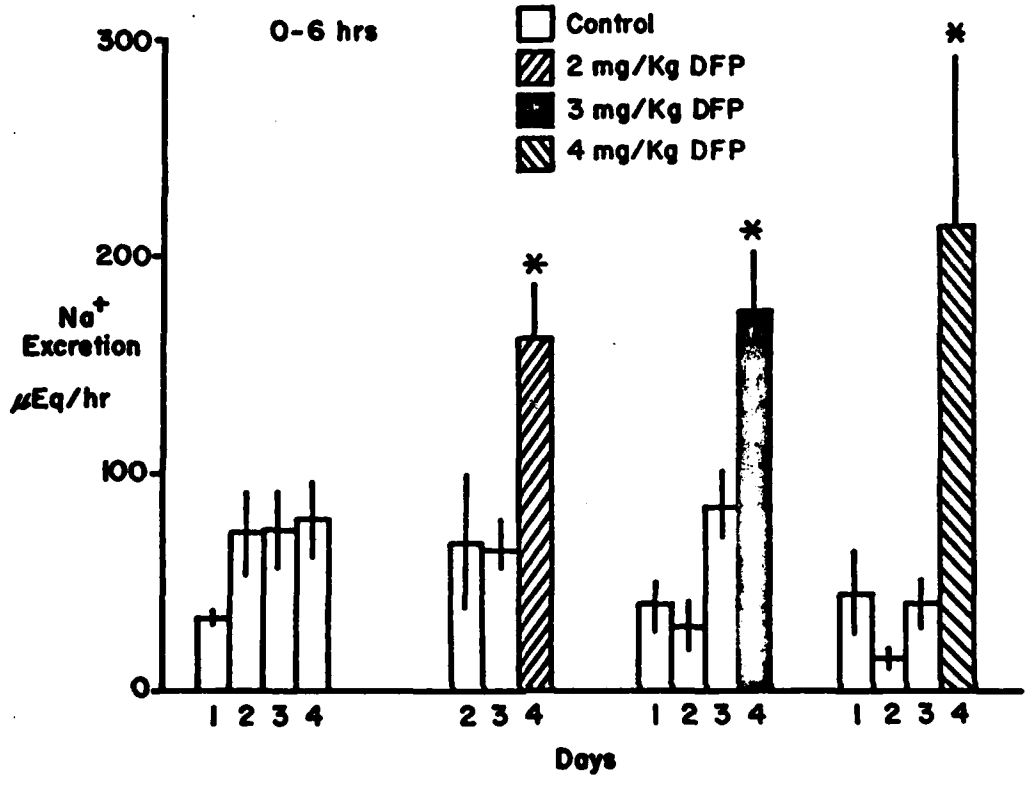


Figure 5. Urinary sodium excretion over 6 hours after DFP administration. The unfilled bars are controls and the filled bars the post-DFP treatment values. The height of the bar is the mean and the vertical line the standard error for N = 4. Statistically significant values ($p < .05$) are indicated by asterisks.

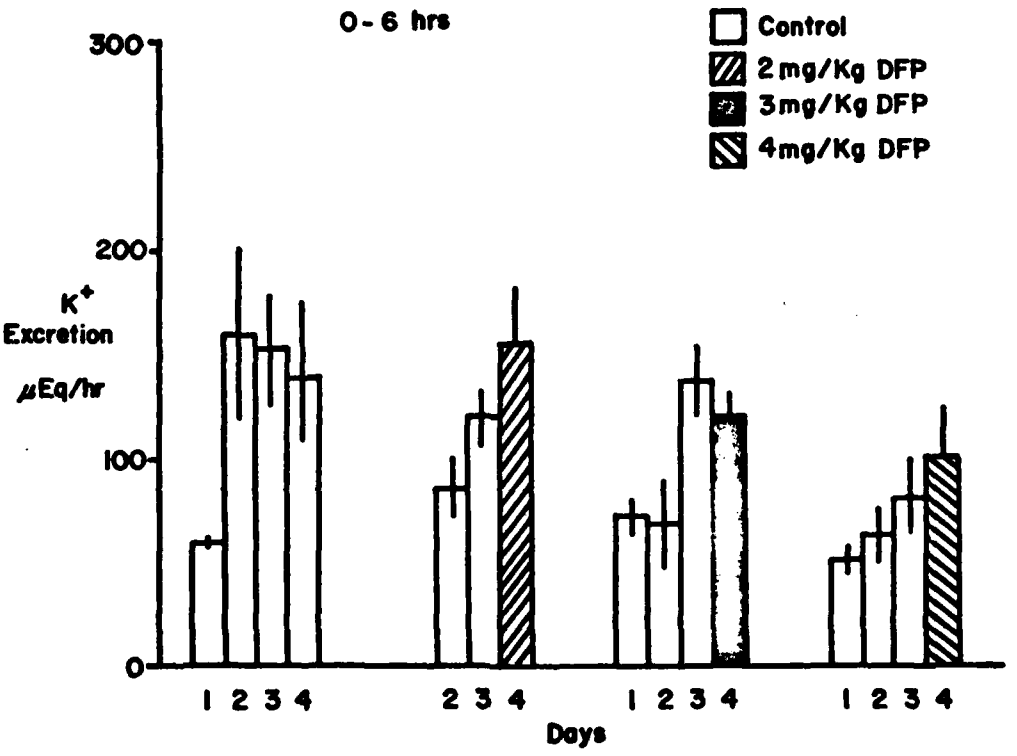


Figure 6. Urinary potassium excretion over 6 hours after DFP administration. The unfilled bars are controls and the filled bars the post-DFP treatment values. The height of the bar is the mean and the vertical line the standard error for N = 4.

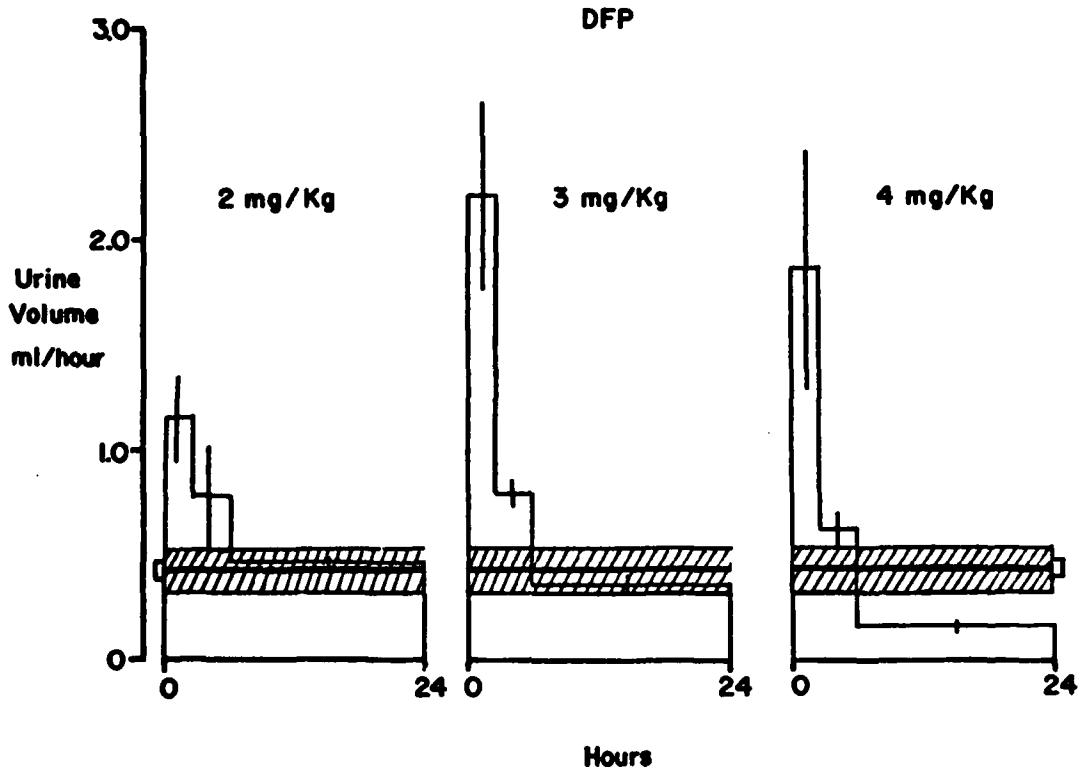


Figure 7. Time course of urinary flow for 24 hours after DFP administration. The height of each bar represents the urine flow averaged over the time indicated. The vertical line is the standard error for N = 4. The heavy line and hatched areas are control values for these animals obtained for 3 days before administration of DFP.

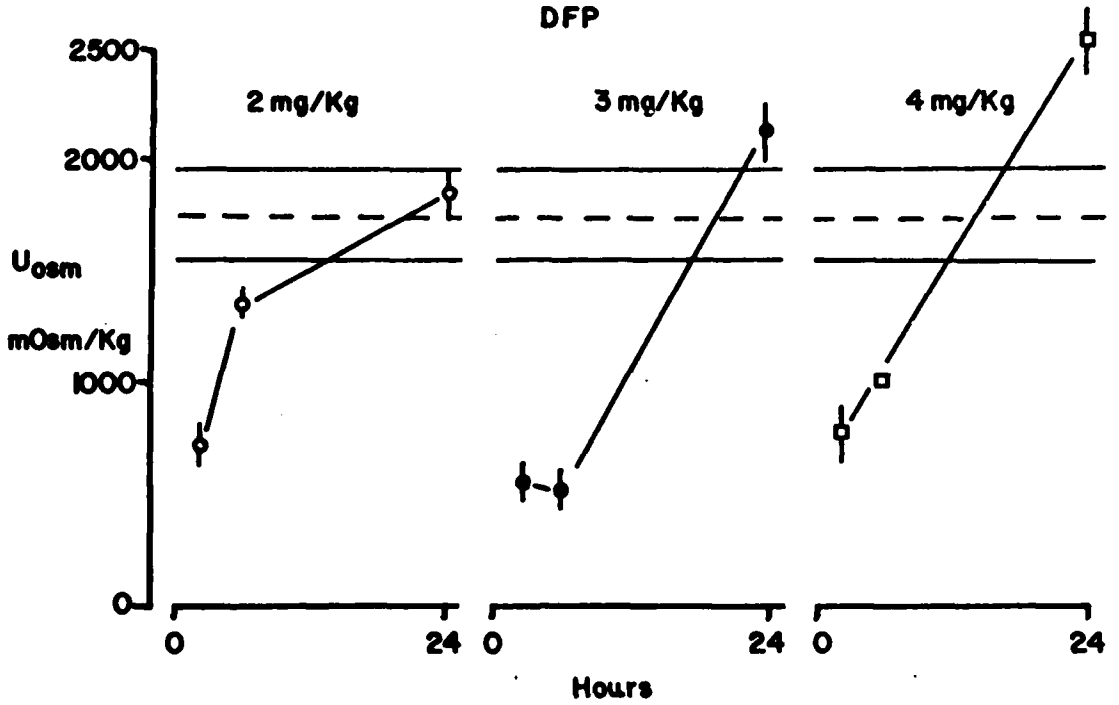


Figure 8. Urinary osmolality measured at three times after DFP administration. Each point is the mean and the vertical line the standard error for N = 4. Statistically significant values ($p < .05$) are indicated by asterisks.

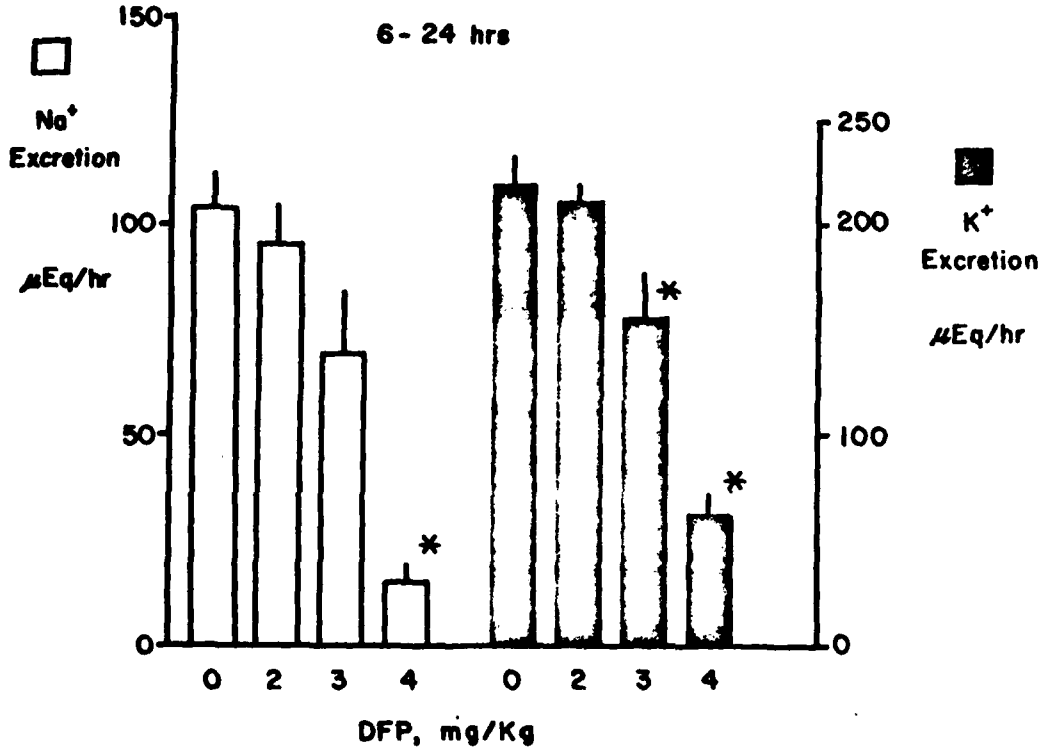


Figure 9. Urinary electrolyte excretion from 6 to 24 hours after DFP administration. The height of each bar is the mean and the vertical line the standard error for N = 4.

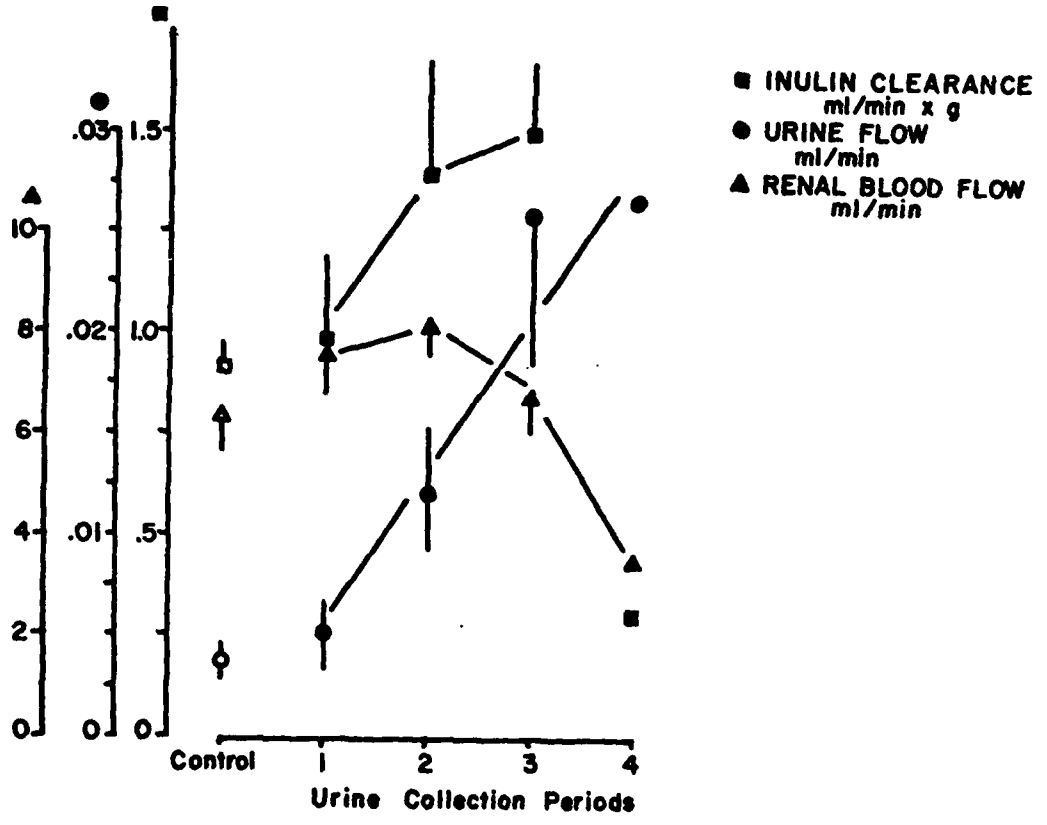


Figure 10. Effect of DFP on renal blood flow (one kidney), inulin clearance and urine flow. Each point is the mean and vertical line the standard error for N = 2 (period 4) or 4 experiments. DFP was administered in doses of 1 mg/kg, SQ, prior to each period 1 through 4.

END

7-87

DTIC